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Research Article

Rapid, Simultaneous Detection of *Clostridium sordellii* and *Clostridium perfringens* in Archived Tissues by a Novel PCR-Based Microsphere Assay: Diagnostic Implications for Pregnancy-Associated Toxic Shock Syndrome Cases

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Clostridium sordellii and *Clostridium perfringens* are infrequent human pathogens; however, the case-fatality rates for the infections are very high, particularly in obstetric *C. sordellii* infections (>90%). Deaths from *Clostridium sordellii* and *Clostridium perfringens* toxic shock (CTS) are sudden, and diagnosis is often challenging. Formalin-fixed, paraffin-embedded (FFPE) tissues usually are the only specimens available for sudden fatal cases, and immunohistochemistry (IHC) for Clostridia is generally performed but it cannot identify species. A clear need exists for a rapid, species-specific diagnostic assay for FFPE tissues. We developed a duplex PCR-based microsphere assay for simultaneous detection of *C. sordellii* and *C. perfringens* and evaluated DNA extracted from 42 *Clostridium* isolates and FFPE tissues of 28 patients with toxic shock/endometritis (20 CTS, 8 non-CTS, as confirmed by PCR and sequencing). The microsphere assay correctly identified *C. sordellii* and *C. perfringens* in all known isolates and in all CTS patients (10 *C. sordellii*, 8 *C. perfringens*, 2 both) and showed 100% concordance with PCR and sequencing results. The microsphere assay is a rapid, specific, and cost-effective method for the diagnosis of CTS and offers the advantage of simultaneous testing for *C. sordellii* and *C. perfringens* in FFPE tissues using a limited amount of DNA.

1. Introduction

Clostridium species are ubiquitous Gram-positive, anaerobic, spore-forming bacteria that are generally found in soil and in the intestinal tract of humans and other animals. *Clostridium sordellii* has been reported to cause a variety of diseases including peritonitis, endocarditis, pneumonia, arthritis, cellulitis, and myonecrosis [1–4]. Fulminant toxic shock syndrome and sepsis among previously healthy persons have been described most often in cases associated with gynecologic infections and neonatal omphalitis [3, 5]. *Clostridium perfringens* is also responsible for a number of clinical conditions in humans ranging from acute food poisoning and enteritis to gas gangrene, enterotoxemia, and endometritis [6–8]. In the last couple of years, several studies reported pregnancy-associated fatal toxic shock syndrome cases due to *C. sordellii* and *C. perfringens* infections [8–10]. Among all *C. sordellii* infections reported in the literature, the overall case fatality ratio is 70%, while, for obstetric infections, it is more than 90% [8]. Pregnancy-associated *C. perfringens* fulminant septicemia also carries a very high mortality rate [8]. Deaths from *C. sordellii* and *C. perfringens* toxic shock (CTS) are sudden, and most patients die from hypotension and multiorgan failure within hours to a few days after the initial presentation.

A presumptive clinical diagnosis of CTS can be challenging and often confounded by nonspecific symptoms, an absence of fever, and the low prevalence of these infections

[10]. A confirmatory diagnosis can only be performed by conventional microbiological isolation and characterization methods including bacterial culture, biochemical analysis, or enzyme-linked immunosorbent (ELISA) assay. However, the fastidious anaerobic growth, variable staining characteristics, and complex biochemical profiles of *Clostridium* species make them difficult to isolate [11, 12]. In addition, for the fatal cases, involving sudden death, unavailability of appropriate specimens in adequate amount poses further challenge. Formalin-fixed, paraffin-embedded (FFPE), archival autopsy or biopsy tissues are often the only specimens available for the fatal cases, and they are unsuitable for microbial methods. For FFPE tissues, generally tissue-based diagnostic methods such as histopathology, special stains, and immunohistochemistry (IHC) are used; however, these methods cannot identify specific *Clostridium* species.

In the last couple of years, we reported detection of *C. sordellii* and *C. perfringens* in FFPE tissues of several fatal obstetrical cases using the conventional, agent-specific toxin genes PCR assays and sequencing [8–10]. These assays can identify species; however, the conventional PCRs and sequencing can be time-consuming, laborious, and expensive. In addition, to perform multiple conventional PCR assays, an adequate amount of patient specimen is required, which is often limiting in cases of sudden death. All the above-mentioned factors highlighted the need to develop a rapid, sensitive, and specific multiplex diagnostic assay such as multiplex PCR. However, traditional multiplex PCR/real time PCR can be challenging because amplification conditions for multiple targets are often incompatible and the combination of several primers, probes, and reagents in a single reaction generally results in loss of sensitivity and specificity for each of the individual target species. In addition, primer dimer formation and spectral overlap of fluorescent labels when using TaqMan or Molecular Beacons may compromise the ability to interpret quantitative data [13]. Luminex xMAP (multianalyte profiling) technology (Luminex Corp., Austin, TX) addresses these issues via application of microsphere-based suspension arrays and allows for multiplexing. Multiplexed microsphere-based detection of bacteria using the Luminex platform has been described previously [14, 15].

The objective of this study was to develop a rapid duplex PCR-based microsphere assay that can simultaneously detect *C. sordellii* and *C. perfringens* in different clinical specimens, including FFPE tissues, and to evaluate its application as a potential diagnostic tool for pregnancy-associated toxic shock syndrome cases in comparison with other tissue-based diagnostic methods, including IHC and conventional PCR.

2. Materials and Methods

2.1. Samples. DNA was extracted from various *Clostridium* isolates ($n = 42$) and FFPE tissues of patients with toxic shock/necrotizing endometritis ($n = 28$) and evaluated by the duplex PCR-based microsphere assay for *C. sordellii* and *C. perfringens.* DNA samples of 42 known *Clostridium* isolates were obtained from the Laboratory Branch, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention (CDC). All 28 cases were submitted to the Infectious Diseases Pathology Branch (IDPB), CDC, from 2004 to 2009 for diagnostic consultation. Clinical and demographic information on the cases were collected when available from medical records. For the confirmation, identification of all the isolates and diagnosis of all the cases were also performed by conventional diagnostic methods. Clinical information as well as conventional diagnostic assay (PCR, sequencing and IHC) results of all *C. sordellii* and all except 2 *C. perfringens* cases were previously published as case reports by our group, and, in this study, these cases were used for validation of the microsphere assay [8–10, 16].

2.2. Conventional Diagnostic Methods. DNA was extracted from FFPE tissues of all the cases using the QIAamp DNA mini kit (QIAGEN) following the tissue extraction protocol as previously described [18]. The DNA samples were evaluated by *C. sordellii* and *C. perfringens* specific conventional PCR assays targeting the lethal toxin and alpha toxin genes and sequencing, as we described before [8]. To monitor the quality of extraction and to verify the presence of amplifiable DNA, samples were also tested for the amplification of the house-keeping gene human beta-globin [19]. FFPE tissues of all the cases were analyzed by routine hematoxylin-eosin (H&E) and special stains to evaluate histopathological changes and IHC for Clostridia species was performed to localize Clostridia antigens in deparaffinized, rehydrated 3 μm sections of tissue samples, as we described previously [20]. For the diagnosis of *C. sordellii* and *C. perfringens* PCR and IHC negative cases, PCR and/or IHC assays for other suspect bacterial agents were also performed [20–25].

2.3. PCR-Based Microsphere Assay

2.3.1. PCR Primers, Oligonucleotide Capture Probes, and Probe Coupling to Microspheres. The primers, probes sequences, gene targets, and amplification product sizes are summarized in Table 1. The primers and probes were synthesized in the Biotechnology Core Facility, CDC, Atlanta, GA. The reverse primers were biotinylated. The probe sequences were labeled at the 5′end with an amino-modified 6-carbon linker and covalently conjugated to individual carboxylated microspheres (Luminex Corp., Austin, TX) using a carbodiimide coupling procedure [26]. A Beckman Coulter Z2 (Hialeah, FL) was used to count conjugated microsphere stocks to determine concentration. Conjugated microspheres were stored in the dark at 4°C in TE buffer. For each probe, a corresponding 5′ biotinylated, complementary oligonucleotide was designed for testing the specificity and effectiveness of microsphere-probe conjugation.

2.3.2. PCR Amplification. DNA samples of all the cases and isolates were evaluated by the microsphere assay. Initially, phospholipase C genes of *C. sordellii* and *C. perfringens* were amplified using a High Fidelity PCR Kit (Roche), 0.3 μM of

Table 1: Oligonucleotide primers and probes used in the duplex microsphere assay.

Primer	Sequence (5′-3′)	Gene target	Product size (bp)
Clostridium sordellii primers and probes			
CSP09-F primer	TGG GAT GAT TGG GAT TAT TCA G	Phospholipase C of *C. sordellii* (Csp)	176 bp
CSP09-BR primer	TCA GTT CCT GCA TAT TCA TTG T	Phospholipase C of *C. sordellii* (Csp)	
CSP09 probe	AGA AGC GAT AAA AAA TTC TCA A	Phospholipase C of *C. sordellii* (Csp)	
Clostridium perfringens primers [17] and probes			
PL3-F primer	AAG TTA CCT TTG CTG CAT AAT CCC	Phospholipase C of *C. perfringens* (plc)	283 bp
PL7-BR primer	ATA GAT ACT CCA TAT CAT CCT GCT	Phospholipase C of *C. perfringens* (plc)	
CP1 probe	TTT AGC AAA ACC TCT TG	Phospholipase C of *C. perfringens* (plc)	

each primer (final concentration), and 5 μL of template DNA sample in a 50 μL final reaction volume, following the manufacturer's instructions. Amplification was carried out on a GeneAmp 9700 thermocycler (Applied Biosystems), using the following cycle conditions: 94°C/2 minutes; 35 cycles of 94°C/20 seconds, 60°C/30 seconds, and 72°C/30 seconds, followed by a final extension of 72°C/5 minutes.

2.3.3. Hybridization of PCR Amplicons to Microsphere Conjugated Probes and Detection. For hybridization, nonpurified biotinylated PCR products were hybridized to the probe-microsphere complexes under optimized reaction conditions in a 96 conical well PCR plate (Costar no. 6509). Each sample was run in triplicate with six reaction blanks per plate. The probe-conjugated microspheres were pulse-vortexed and sonicated for at least 40 seconds, and a working microsphere mixture was prepared by adding calculated volumes of both sets of conjugated microspheres (*C. sordellii* and *C. perfringens*) to 1.5X TMAC buffer (4.5 M tetramethyl ammonium chloride, 75 mM Tris-HCl, pH 8.0, 6 mM EDTA, and 0.15% sarkosyl) to achieve a concentration of 1500 microspheres per set per well.

In each sample well of the PCR plate, 30 μL of microsphere mix and 20 μL of sample mix, composed of 1–5 μL biotinylated PCR amplification product in TE buffer (pH 8.0), were added. Reaction blanks contained only microsphere mix and TE buffer. The PCR plate was covered, and incubated in a thermocycler at 95°C for 5 minutes, followed by 48°C for 30 minutes. After incubation, the reactions were transferred to a filter plate and washed two times with 1X TMAC buffer using a vacuum manifold. Reporter mixture that contained a fresh 1 : 100 dilution of streptavidin-R-phycoerythrin (SA-PE; Molecular Probes, Eugene, OR) in 1X TMAC buffer was added to each well (75 μL), carefully mixed by hand pipetting and transferred to a PCR plate. The plate was covered, and the samples were heated to 48°C for 15 minutes and analyzed at 48°C on the Bio-Plex 200 system. BioPlex Manager Software v. 5.0 (Bio-Rad Hercules, CA) was used for data acquisition. The hybridization of biotinylated PCR amplicons to the probe sequences on the respective microsphere populations generated the Median Fluorescence Intensity (MFI), which represented the SA-PE fluorescence of 100 biotinylated amplicon-bound microspheres. The reported signal for each probe was obtained by subtracting the mean MFI of the blanks from the MFI values of the samples. The MFI value greater than 4 times the mean MFIs of the negative control isolates was designated as the cut-off value for positive signal detection.

3. Results

Organism specific PCR and sequencing identified *C. sordellii* in 10 cases, *C. perfringens* in 8 cases, and mixed infection of *C. sordellii* and *C. perfringens* in 2 cases of CTS. In the non-CTS cases, PCR and/or IHC assays detected *C. difficile* in 2 cases and *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Neisseria meningitides*, *Neisseria gonorrhoeae*, or *Bacillus cereus* was identified in one case each. Demographic and clinical information, pathologic findings, results of conventional diagnostic assays, including organism specific PCR, sequencing, and IHC assay, and the duplex microsphere assay for all the CTS cases are listed in Table 2.

The mean age for the *C. sordellii* patients was 24 years and 33 years for the *C. perfringens* patients. All cases, except one *C. sordellii* case, were fatal. Of 10 *C. sordellii* cases, 8 were medical abortion cases, 1 was a spontaneous abortion case, and 1 had no pregnancy association but was associated with a gynecological procedure. Of 8 *C. perfringens* cases, 3 were medical abortion, 4 were postpartum cases, and 1 was a spontaneous abortion case. Of 42 isolates included in the study, 9 each were isolates of *C. sordellii* and *C. perfringens* and 24 were various other *Clostridium* species isolates (4 *C. difficile* and 5 each *C. butyricum, C. septicum, C. sporogenes, C. tetani*), as confirmed by conventional diagnostic assays.

The duplex microsphere assay correctly identified *C. sordellii* and *C. perfringens* in all 18 known isolates of *C. sordellii* and *C. perfringens* (9 each). All 24 other Clostridia isolates were negative by the assay (Table 3). Figure 1 shows the MFI readings for the *C. sordellii* and *C. perfringens* isolates. The MFI readings for non-clostridia isolates (negative controls) ranged from 0 to 58. Thus, the microsphere assay showed 100% analytical specificity for *C. sordellii* and *C. perfringens*. The detection limits of the microsphere and conventional PCR assays were determined by preparing serial

Table 2: Demographic, clinicopathological findings, and case-by-case comparison of IHC, PCR, and microsphere assay results.

Case no.	Age	Association with pregnancy	Outcome	Tissue tested	Pathological findings	Clostridia IHC	*C. sordellii* PCR	*C. perfringens* PCR	Microsphere assay
1	18	Medical abortion	Fatal	Uterus	Necrotizing endomyometritis	Positive	Positive	Negative	Positive for *C. sordellii*
2	22	Medical abortion	Fatal	Uterus	Necrotizing endomyometritis	Positive	Positive	Negative	Positive for *C. sordellii*
3	34	Medical abortion	Fatal	Uterus	Necrotizing endomyometritis	Positive	Positive	Negative	Positive for *C. sordellii*
4	21	Medical abortion	Fatal	Uterus	Necrotizing endomyometritis	Positive	Positive	Negative	Positive for *C. sordellii*
5	26	Medical abortion	Fatal	Endometrium	Necrotizing endomyometritis	Positive	Positive	Negative	Positive for *C. sordellii*
6	25	Spontaneous abortion	Non-fatal	Placenta	Severe acute chorioamnionitis	Positive	Positive	Negative	Positive for *C. sordellii*
7	32	No association	Fatal	Uterus	Necrotizing endometritis	Positive	Positive	Negative	Positive for *C. sordellii*
8	18	Medical abortion	Fatal	Unknown	Necrotizing endometritis	Positive	Positive	Negative	Positive for *C. sordellii*
9	29	Medical abortion	Fatal	Uterus	Necrotizing endometritis	Positive	Positive	Negative	Positive for *C. sordellii*
10	21	Medical abortion	Fatal	Decidual and chorionic tissue	Acute inflammation and necrosis	Positive	Positive	Negative	Positive for *C. sordellii*
11	24	Medical abortion	Fatal	Uterus	Necrotizing endomyometritis	Positive	Negative	Positive	Positive for *C. perfringens*
12	28	Medical abortion	Fatal	Uterus	Necrotizing endomyometritis	Positive	Negative	Positive	Positive for *C. perfringens*
13	40	Postpartum	Fatal	Uterus	Acute endomyometritis	Positive	Negative	Positive	Positive for *C. perfringens*
14	41	Postpartum	Fatal	Uterus	Necrotizing endometritis	Positive	Negative	Positive	Positive for *C. perfringens*
15	37	Medical abortion	Fatal	Uterus	Necrotizing endometritis	Negative	Negative	Positive	Positive for *C. perfringens*
16	26	Postpartum	Fatal	Uterus	Necrotic and hemorrhagic uterus	Negative	Negative	Positive	Positive for *C. perfringens*
17	41	Spontaneous abortion	Fatal	Uterus	Endomyometritis and sepsis	Positive	Negative	Positive	Positive for *C. perfringens*
18	32	Postpartum	Fatal	GI	Necrotizing enteritis	Positive	Negative	Positive	Positive for *C. perfringens*
19	16	Medical abortion	Fatal	Appendix	Acute appendicitis and peritonitis	Positive	Positive	Positive	Positive for both
20	40	No association	Fatal	Cervix	Acute cervicitis and endometritis	Positive	Positive	Positive	Positive for both

dilutions of *C. sordellii* and *C. perfringens* isolate DNA for testing by the assays. The detection limit for the conventional PCR assays was 2 ng/μL for the starting DNA sample, while the detection limit of microsphere assay was 1.4 ng/μL. Thus, clinical sensitivity of the duplex microsphere assay was slightly higher than the single-plex conventional PCR assays.

The microsphere assay also accurately detected *C. sordellii* and *C. perfringens* in FFPE tissues of all confirmed CTS cases (10 *C. sordellii*, 8 *C. perfringens*) as summarized in Tables 2 and 4. Figure 2 shows the MFI readings of these CTS cases. The assay was also able to identify both *C. sordellii* and *C. perfringens* in 2 mixed infection cases. FFPE tissues from 8 patients with non-CTS bacterial infections were negativewith MFI readings ranging from 0 to 23. These results demonstrate that the microsphere assay was 100% concordant with the conventional PCR and sequencing results.

Table 3: Microsphere assay results of *Clostridium* species isolates.

Clostridium species	Number of isolates tested	Number of positive isolates	
		For *C. sordellii*	For *C. perfringens*
Clostridium sordellii	9	9/9 (100%)	0/9 (0%)
Clostridium perfringens	9	0/9 (0%)	9/9 (100%)
Clostridium difficile	4	0/4 (0%)	0/4 (0%)
Clostridium butyricum	5	0/5 (0%)	0/5 (0%)
Clostridium septicum	5	0/5 (0%)	0/5 (0%)
Clostridium sporogenes	5	0/5 (0%)	0/5 (0%)
Clostridium tetani	5	0/5 (0%)	0/5 (0%)

Table 4: Microsphere, PCR, and IHC assay results of CTS cases.

Clostridium cases (no. of cases)	Positive for *C. sordellii*			Positive for *C. perfringens*		
	IHC*	PCR**	Microsphere assay	IHC*	PCR**	Microsphere assay
C. sordellii ($n = 10$)	10	10	10	10	0	0
C. perfringens ($n = 8$)	6	0	0	6	8	8
C. sordellii and *C. perfringens* coinfection ($n = 2$)	2	2	2	2	2	2

*IHC assay is specific for Clostridia but cannot speciate further.
**Conventional PCR assay targeting the phospholipase C gene of *C. sordellii* and *C. perfringens.*

The *Clostridium* IHC was positive in all except for 2 *C. perfringens* cases. Figure 3(a) illustrates the hemorrhage, inflammation, and necrosis of the endometrium and abundant Gram-positive bacilli in a positive *C. sordellii* case. IHC also detected Clostridial antigens inside the inflammatory cells present in the necrotic endometrial tissues (Figure 3(b)) and in myometrial blood vessels of the positive CTS cases.

4. Discussion

This study describes the development of a duplex PCR-based microsphere assay using a suspension array technology, the Luminex xMAP, for rapid, simultaneous detection of *C. sordellii* and *C. perfringens.* Although DNA assays developed on the Luminex platform have been used for identification and genotyping of infectious agents such as *Mycobacterium*, *Escherichia coli*, *Salmonella*, *Cryptosporidium, Listeria, Candida*, and various respiratory bacteria and viruses [14, 15, 26–29] in serum and other clinical specimens, to our knowledge, this represents the first report of the application of this technology for the diagnosis of *Clostridium* infections, particularly in the FFPE archival tissues. This duplex microsphere assay can have important implications for the premortem and postmortem diagnosis of pregnancy-associated CTS cases.

In recent years, *C. sordellii* and *C. perfringens* have been found to be associated with severe or fatal toxic shock syndrome in both postpartum and postabortive women [8–10]. Although pregnancy and gynecologic infections due to *C. sordellii* and *C. perfringens* are rare, they progress so rapidly that death often precedes diagnosis. Early confirmatory diagnosis before the development of irreversible toxic shock is critical for implementation of treatment measures and support of epidemiologic investigations. However, presumptive diagnosis of CTS often relies on vague clinical manifestations and is quite challenging. Also, the isolation of *Clostridium* species is very difficult. Only molecular approaches guarantee identification to the species level, which is important for the selection of treatment as well as for the identification of the source of infection [9]. Generally, several diagnostic assays for different organisms have been performed on the premortem biopsy samples for differential diagnosis, but selecting individual assays in sequential order can delay diagnosis and treatment as well as prolong hospitalization. On the other hand, simultaneous testing by individual assays for each organism can be expensive and requires large volumes of patients' samples, which is usually difficult to obtain, particularly for premortem diagnosis. Even for the fatal cases, autopsies are often not performed and the FFPE archival biopsy tissues are the only specimens available. The microsphere assay addresses these issues and is particularly useful for the rapid, accurate, and simultaneous identification of *C. sordellii* and *C. perfringens* in FFPE tissues using only a small volume of starting material. Another added advantage of this assay is the low cost. The total cost of one test using this approach is less than 20 cents excluding costs for lab personnel and equipment. On the other hand, the traditional PCR and automated DNA sequencing methods cost at least $4 per test.

In this study, we compared conventional PCR assays and microsphere assay results for various Clostridia stock

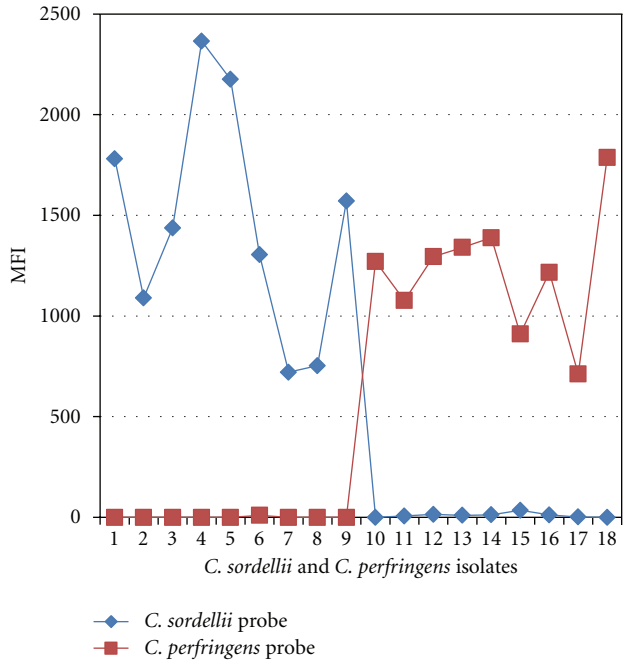


Figure 1: Identification of *C. sordellii* and *C. perfringens* isolates by the microsphere assay. Isolates 1–9: *C. sordellii* isolates. Isolates 10–18: *C. perfringens* isolates. Note higher MFI values/signals of *C. sordellii* and *C. perfringens* probes for the respective isolates only, indicating specificity of the probes.

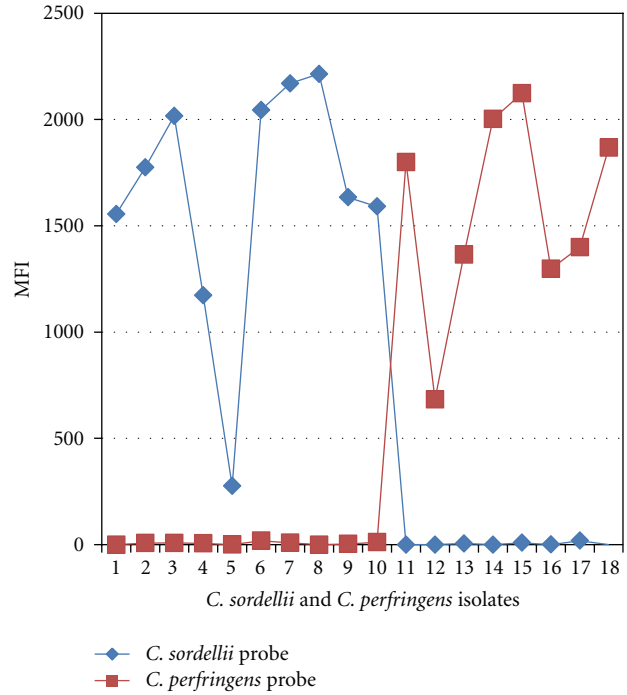


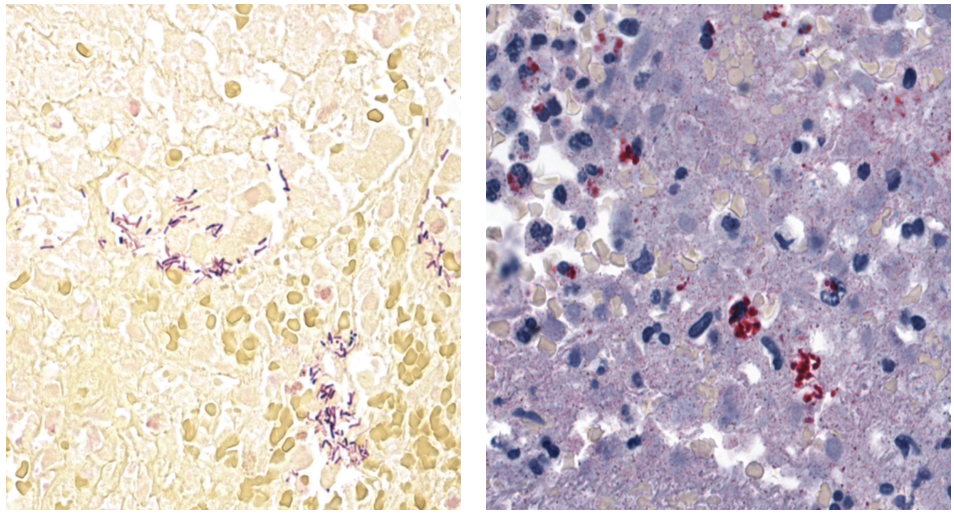
Figure 2: Identification of *C. sordellii* and *C. perfringens* in confirmed CTS cases by the microsphere assay. Cases 1–10: *C. sordellii* cases confirmed by conventional PCR and sequencing. Cases 11–18: *C. perfringens* cases confirmed by conventional PCR and sequencing. No cross-reactivity was noted.

strains (isolates) and FFPE tissue specimens from confirmed pregnancy or gynecologic procedure-associated CTS cases to establish analytic sensitivity and specificity of the assay. For the microsphere assays, direct DNA hybridization was used in conjunction with short probes and TMAC buffer to produce a stringent assay. Tetramethyl ammonium chloride (TMAC) binds to the A-T rich regions of the genome and significantly reduces the difference in the melting temperature between the A-T and G-C pairs. The phospholipase C gene was targeted for the primers and probes because of its presence in all types and strains of *C. sordellii* and *C. perfringens*. Our results clearly demonstrate the high level of clinical sensitivity and specificity of the microsphere assay for the pathogens examined with 100% concordance with the conventional PCR assay results for all clinical cases and isolates. In addition, the microsphere assay was able to detect mixed infections of *C. sordellii* and *C. perfringens* and showed no cross-reactivity with any other organisms tested. The average MFI value for the *C. sordellii* cases was 1645 and for *C. perfringens* cases was 1567. No correlation was seen between the MFI values of *C. sordellii* and *C. perfringens* cases and the duration of illness, type of infection (medical abortion versus postpartum), IHC results, or any other clinical feature.

We also performed histopathological evaluation and Clostridia IHC on the tissues to define the pathology and to detect Clostridial antigens in the areas of inflammation and pathology. This information is particularly important because *Clostridium* species are present in the vaginal tract of 4–18% of healthy women [9, 30, 31]. We detected Clostridial antigens by IHC in the areas of pathology, particularly in the necrotic endometrial tissue in all confirmed CTS cases, except for 2 *C. perfringens* cases. In these IHC negative cases, MFI values of the microsphere assay were quite good (above 1200) and the conventional PCR assays were also positive. The reason for this discrepancy may be the lower sensitivity of the IHC assay in comparison to the PCR and microsphere assay or clearance of antigens by the host immune system.

5. Conclusions

The duplex microsphere assay is a rapid, sensitive, specific, and cost-effective method for the diagnosis of pregnancy-associated CTS cases and offers the advantage of simultaneously testing for *C. sordellii* and *C. perfringens* in FFPE tissues using a limited amount of DNA. The assay can be an excellent diagnostic tool for the identification of mixed infections. This technology also allows for further multiplexing of additional pathogens associated with toxic shock syndrome that could be of great value for epidemiologic investigations. In addition, the combination of the microsphere assay and IHC for the analysis of Clostridial DNA and antigens together in the tissues of fatal cases can provide an insight into the disease pathogenesis, improve detection, and have important implications for the diagnosis of CTS. Early recognition and confirmatory diagnosis of CTS, along with an aggressive surgical approach and appropriate antimicrobial therapy, can



(a) (b)

Figure 3: (a) Abundant-gram-positive bacilli in the necrotic endometrial tissues (Gram's stain). (b) Clostridial antigens (red staining) inside the inflammatory cells present in the necrotic endometrial tissues (IHC using polyclonal anti-clostridium species antibody).

decrease the mortality associated with this syndrome that occurs primarily among young, otherwise healthy women.

Disclaimer

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Conflict of Interests

The authors cite no relationships or support, which might be perceived as constituting a conflict of interests.

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